

**IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA**

STATE OF OKLAHOMA, ex rel,
W. A. DREW EDMONDSON,
in his capacity as ATTORNEY GENERAL
OF THE STATE OF OKLAHOMA,
and OKLAHOMA SECRETARY
OF THE ENVIRONMENT
C. MILES TOLBERT, in his capacity as
the TRUSTEE FOR NATURAL RESOURCES
FOR THE STATE OF OKLAHOMA,

Plaintiffs,

vs.

TYSON FOODS, Inc.,
TYSON POULTRY, INC.,
TYSON CHICKEN, INC.,
COBB-VANTRESS, INC.,
AVIAGEN, INC.,
CAL-MAINE FOODS, INC.,
CAL-MAINE FARMS, INC., CARGILL, INC.,
CARGILL TURKEY PRODUCTION, LLC,
GEORGE'S, INC., GEORGE'S FARMS, INC.,
PETERSON FARMS, INC.,
SIMMONS FOODS, Inc.
WILLOWBROOK FOODS, INC.

Defendants.

Case No. 4:05-cv-00329-GKF-SAJ

EXPERT REPORT OF VALERIE J. HARWOOD, Ph.D.

55. *Nested Sybr green PCR*. When the PLB concentration was below detection limit in the QPCR assay, a nested variant of this assay (which is presence-absence, rather than quantitative) was used to determine if lower levels of the PLB were present. In this case DNA extracted from the environmental samples was first amplified by conventional PCR using universal bacterial (16S rRNA) primers. This primary amplification step was followed by a secondary amplification step with the PLB primers (the LA 35 set). The identity and purity of the PCR product was always checked by conducting a melting curve analysis. This nested Sybr green procedure allowed detection of the PLB in many samples in which the PLB was at too low a concentration to quantify. Of 40 total soil samples collected from fields that received land-applied poultry litter, 38 had detectable levels of the PLB. Of 187 water samples (including 3 reference unimpacted samples) 99 had PLB levels below the detection limit, but 88 water samples had detectable levels of the PLB, including 1 geoprobe (shallow groundwater) sample (GPGW-10-4-11-30-06). A total of 3 spring or groundwater samples had detectable or quantifiable concentrations of the PLB, demonstrating transport of poultry waste in the subsurface. Furthermore, two of the samples that contained quantifiable concentrations of the PLB (HFS16-BF2-03-8-27-05 and HFS22-BF2-01-8-1-06) were base flow samples, which consist mainly of groundwater. Figures 5 and 6 show the results of nested Sybr green PCR testing for the PLB in water and soil samples, respectively. Sites at which the PLB was detected, but was too low to quantify by QPCR are designated by black triangles.

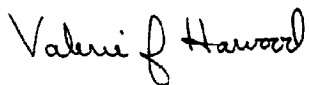
VI. CONCLUSIONS

56. Testing of poultry litter, soils upon which poultry litter has been applied, and edge-of-field samples collected from ditches during runoff conditions all show high levels of fecal indicator bacteria, some of which approach the levels expected in raw sewage. When these bacteria reach the extensive network of IRW tributaries, they become dominant contributors to the fecal indicator bacteria loads that impair the use of the Illinois River and its tributaries as recreational waters. The fecal indicator bacteria concentrations observed in the IRW tributaries, including those that receive extensive recreational use, are not characteristic of those in rural areas that are unimpacted by fecal contamination; rather, they are similar to areas that are extensively impacted by sewage or large-scale animal farming. The pathogenic microorganisms that are excreted in poultry feces and land-applied on contaminated poultry litter can impact the health of those who use the river for recreation, and also penetrate into the groundwater and contaminate the area's rural drinking water source. Sampling of IRW surface

water, groundwater, soil and sediments has revealed a unique chemical and bacterial signature that indicates contamination by poultry; and this signature is not present in areas that are remote from poultry operations. The finding that a poultry litter-specific biomarker (PLB) is found in all environmental compartments tested in the IRW, from soil samples to edge-of-field samples to surface water and groundwater, firmly links a dominant portion of the indicator bacteria contamination to poultry waste, which is well known to contain important human pathogens such as *Salmonella* and *Campylobacter*. Thus, the disposal of poultry waste by land application in the IRW presents a substantial, serious and immediate threat to human health.

57. If land application of poultry litter continues in the IRW, the loading of bacteria and particulate matter, which contributes to water turbidity, will continue. Much of this particulate matter settles out in stream bottoms and forms a habitat where the microbial contaminants can survive for long time periods – on the order of months or longer. The quality of surface water and groundwater in the IRW will continue to decline and the threat to human health will remain or increase. If land application of poultry litter ceases a major source of microbial contamination to the IRW will be removed. Once land application ceases and rain events over a season scour the contaminated soils and sediments, microbial water quality should substantially improve and the threat to human health will substantially decrease.

58. My opinions in this matter are my own, and do not reflect an official view of the University of South Florida.



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